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Prevalence of Feline Panleukopenia Virus in Stray and Household Cats in Seoul, Korea

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Abstract : We investigated the prevalence of feline panleukopenia virus (FPV) in stray and household cats in different regions of Seoul, Republic of Korea. Blood samples were collected from a total of 200 cats (100 stray cats and 100 household cats) and examined by polymerase chain reaction (PCR). The overall prevalence of FPV was 2%. Among test-positive cats, 3% (3/100) were stray cats and 1% (1/100) was a household cat. The incidence of FPV was higher in juvenile cats (< 1 year, 1.5%) than in adult cats (> 1-year-old, 0.5%). The FPV-positive rates of healthy infected cats and sick cats were 1.9% (3/156) and 2.2% (1/44), respectively. We found the positive rate of vaccinated and unvaccinated cats to be 1.3% (1/77) and 2.4% (3/123), respectively. Unlike antibody tests, FPV antigen tests detected current infections in stray and household cats. Therefore, these tests can help in disease diagnosis and treatment. To our knowledge, our study is the first to survey the prevalence of FPV in different cat populations across Seoul. We found a high prevalence of FPV infection in stray and juvenile cats. Therefore, proper vaccination and surveillance are important to prevent FPV outbreaks.

Key words **:** Feline panleukopenia virus, polymerase chain reaction, Seoul, stray cats, household cats.

Introduction

In our country, feline panleukopenia virus (FPV) infection is one of the most important viral diseases in cats. FPV is a disease characterized by the single-stranded deoxyribonucleic acid (DNA)-containing feline parvovirus that affects all members of the family Felidae (18). It is most commonly transmitted by direct contact of susceptible animals with infected cats or their secretions. The severity of clinical signs depends on age, immune status, nutritive condition, and concurrent infections (22). According to the previous reports (22), vomiting usually develops 1-2 days after the onset of fever and is typically bilious and unrelated to meals. Diarrhea may begin slightly later but is not always present. Terminal cases are hypothermic and may develop septic shock and disseminated intravascular coagulation. Morbidity and mortality is highest in young kittens up to 1 year of age (1,5). It was reported that mortality is 25-90% for acute panleukopenia and up to 100% in peracute infections (23). A presumptive diagnosis is usually based on clinical signs of acute gastroenteritis in young susceptible (unvaccinated) cats with systemic involvement and profound panleukopenia (total leukocyte count < 500/μL). Diagnosis can be confirmed by detection of the FPV antigen, serologic diagnosis (paired neutralizing antibody titers), immunofluorescent antibody testing, polymerase chain reaction (PCR) assay, and virus isolation (6). FPV prevalence has been surveyed in many countries, including Iran (9), Canada (8), East Africa (7), Spain (16), Vietnam (19), Central West Saudi Arabia (21), and Brazil (15). However, epidemiological FPV surveys of only a few cats have been conducted in Korea (2,12,13), and, to our knowledge, no study has compared the prevalence of FPV infections in stray and household cats in the same area.

Prevalence data are necessary to define prophylactic, management, and therapeutic measures for stray and household cats. The purposes of this study were to determine the prevalence of FPV infection among stray and household cats in Seoul via PCR analysis and to identify risk factors for positivity.

Materials and Methods

Selection procedure and data collection

Informed consent was given for cats to enter the study, and the institutional animal care and use committee (IACUC) of Konkuk University approved the study protocol. The survey was conducted in 200 cats in Seoul, South Korea, divided into 2 groups based on their origins (100 stray and 100 household cats). Both groups were of the same species (*Felis catus*) but differed in lifestyle, as the stray cats were unowned. Household cats were selected randomly from cats referred to the Veterinary Medical Teaching Hospital (VMTH) of Konkuk University and 14 local animal hospitals in Gangseo-

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Fig 1. Areas surveyed for feline panleukopenia virus (FPV) infection in stray and household cats in Seoul, Korea (blue rectangles). Red rectangles represent areas with FPV-positive samples.

gu, Kwangjin-gu, Jungrang-gu, Kangnam-gu, Kangdong-gu, and Mapo-gu in Seoul (Fig 1). Stray cats were also selected from Gangseo-gu, Kwangjin-gu, Jungrang-gu, Kangnam-gu, Kangdong-gu, and Mapo-gu but not from the VMTH (Fig 1). All samples were collected between June and November 2012. Blood samples of household cats were collected when they were admitted to a veterinary clinic by their owners for regular health checkups and procedures such as neutering, boarding, grooming, and vaccination. Blood samples from stray cats were received from other veterinary clinics in the same areas from which the household cat samples were obtained, through the local government's Trap, Neuter, and Return program, the purpose of which is to reduce the number and control the natural reproduction rates of urban stray cats. All the cats included in the study were assessed for sex, age, and breed; and vaccination status was also assessed for household cats. Household cats were categorized as "unvaccinated" if they had never received a vaccine against FPV and categorized as "1 or more vaccines administered" if they had ever received 1 or more vaccines against panleukopenia. Age was estimated by evaluating the annual layers of teeth and bone (20). Stray cats were classified according to age as follows: < 1, 1-2, 2-4, and > 4 years of age.

Complete blood count and polymerase chain reaction

Peripheral blood samples (0.5-0.7 mL) were obtained by cephalic or jugular venipuncture and collected into ethylenediaiene tetraacetic acid (EDTA) anticoagulant tubes for viral DNA extraction by PCR. The tubes were kept on ice while being transported. Blood samples were tested for complete blood counts and then centrifuged for 15 min at 890 × *g* at 4°C (Centrifuge MICRO 17TR, Hanil Science Industrial Co., Ltd., Inchon, Korea). Blood plasmas were collected and stored at −80°C until they were analyzed. Nucleic acids were extracted from specimens using the QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. DNA concentrations were measured using a NanoDrop 2000 spectrometer (Thermo Scientific, Waltham, MA, USA). The mean DNA concentration of each blood sample was 75 ng (range, 35-93 ng). The DNA was stored at −80°C until PCR was performed. A commercial vaccine (Felocell CVR-C; Pfizer Animal Health, New York, NY, USA) was extracted as described above and used as a positive control; sterile distilled water without nucleic acids was a negative control for both nucleic acid extraction and PCR.

PCR was performed to identify FPV-positive samples. Oligonucleotide primers (FPV forward: 5'-GCTTTAGATGATACTCATGT-3' and FPV reverse: 5'- GTAGCTTCAGTAATATAGTC-3') were derived from the published sequence of the FPV VP2 capsid protein gene (2) to amplify a 698-base pair (bp) fragment from nt 3113 to 3810 (GenBank accession No. D78585). These primers were used to perform FPV diagnostic PCR using a Maxime PCR PreMix Kit (Intron, Seoul, Korea). PCR reactions were cycled 30 times with denaturation at 94°C for 30 s, followed by primer annealing at 55°C for 2 min and extension at 72°C for 30 s. The 20 μL of amplified PCR products was electrophoresed on a 1.5% agarose gel and stained with ethidium bromide. Appropriate molecular size markers (100-bp DNA ladder; Bioneer, Daejeon, Korea) were placed in gel lanes adjacent to the samples. The positive control included extracted nucleic acid from the commercial vaccine strains. The negative controls consisted of all PCR reagents except the template and were included in each reaction.

Results

Our study population consisted of 100 stray cats (45 intact males and 55 intact females) and 100 household cats (34 castrated males, 22 intact males, 20 spayed females, and 24 intact females). The stray cats were mostly domestic short hair, whereas the household cats were purebreds (58%) and domestic short hairs (42%). The age range for all the cats was 0.3-14 years (median, 3.88 years). The stray cats (median, 3.1 years; range, 0.3-11 years) were significantly younger than the household cats (median, 4.2 years; range, 0.3-14 years). Vaccination status was unknown for the stray cats. Of the household cats for which vaccination status was known, 23 (23%) were not vaccinated; the remaining 77 (77%) had received at least 1 vaccine. The FPV prevalence results are shown in Tables 1 and 2 and Figs 1-3. The total number of positive FPV samples was 2% (4 cats) in the 200 samples tested. Among the test-positive cats, 3 (3% of 100) were stray cats and 1 (1% of 100) was a household cat. Of these FPV-positive cats, 3 were strays and 1 was a household cat. The FPV-positive breeds were domestic short hair (1.5%, 3/200) and Scottish Fold (0.5%, 1/200; Table 1). We found no significant difference in the positive rate between the males (1%, 2/200) and females (1%, 2/200). The age distribution of positive cases was 3 cats < 1 year and 1 cat > 1 year (Table 2). The highest FPV-positive rate was observed in cats < 1 year. The highest positive rates of FPV infection were noted in Gangseo (1.5%, stray cats; Fig 2) and Gwangjin (0.5%, household cats; Fig 3) via PCR. The positive rate of healthy cats was 1.9% (3/156), and the positive rate of sick cats was 2.2% (1/44). The positive rate of vaccinated cats was 1.3% (1/77), and the positive rate of unvaccinated cats was 2.4% (3/123). The single positive-sample household cat presented with severe leukopenia (630/μL; reference range, 6,000-19,000/μL), but the 3 positive stray cat samples had leukocyte counts within the reference range (median, 16,820/μL).

Table 1. Breed distribution and FPV status among the stray and household cats

Living environment	Breed	Number of samples (%)	Number of positives (%)
Stray cats	Korean Short Hair	100(100)	3(1.5)
Household cats	Korean Short Hair	42(42)	0
	Persian	14(14)	0
	Siamese	9(9)	0
	Russian Blue	5(5)	0
	Scottish Fold	5(5)	1(0.5)
	Turkish Angola	5(5)	0
	American Short Hair	3(3)	0
	Bengal	2(2)	0
	Mix	2(2)	0
	British Short Hair	1(1)	0
	Abyssinian	1(1)	0
	Norwegian Forest Cat	1(1)	0
Total		200(100)	4(2)

Table 2. Stray and household cat group characteristics and areas surveyed within Seoul

Designation		Number of stray cats	Number of household cats	Number of positive stray cats (%)	Number of positive household cats (%)
Age (years)	< 1	18	15	3(1.5%)	0
	1~2	21	10	0	1(0.5%)
	2~4	35	33	0	0
	> 4	26	42	0	0
Total		100	100	3(1.5%)	1(0.5%)
Sex	Male	45	56	2(1%)	0
	Female	55	44	1(1%)	1(0.5%)
Total		100	100	3(1.5%)	1(0.5%)
Areas	K.S	69	9	3(1.5%)	0
	K.J	10	35	0	1(0.5%)
	J.R.	1	34	0	0
	K.N.	15	9	0	0
	K.D.	2	9	0	0
	M.P.	3	4	0	0
Total		100	100	3(1.5%)	1(0.5%)

K.S., Kangseo; K.J., Kwangjin; J.R., Jungrang; K.N., Kangnam; K.D., Kangdong; M.P., Mapo

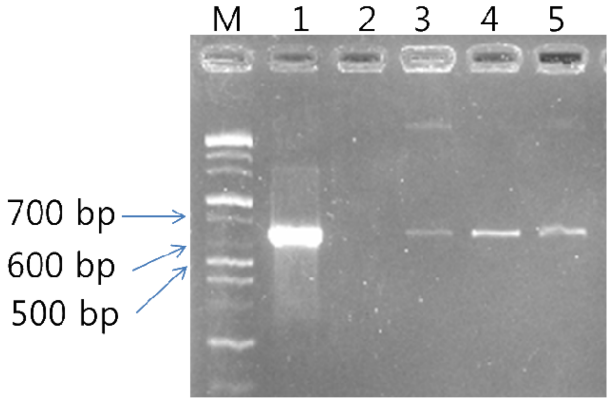

Fig 2. Agarose gel electrophoresis of a 698-base pair (bp) region of the FPV VP2 capsid protein gene. The first lane (M) contains a 100-bp DNA ladder. Lane 1 is a FPV-positive control, lane 2 is a negative control, and lanes 3-5 contain FPV-positive samples from stray cats.

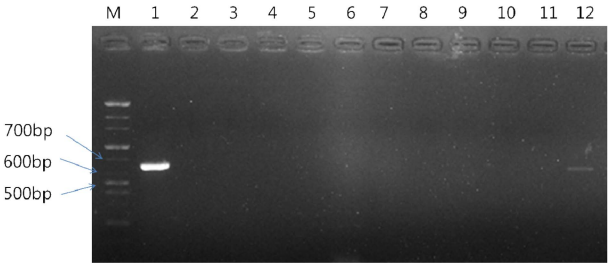

Fig 3. Agarose gel electrophoresis of the 698-base pair (bp) region of FPV VP2 capsid protein gene. The first lane (M) contains a 100-bp DNA ladder. Lane 1 is a positive control, lane 2 is a negative control, lanes 3-11 are FPV-negative samples from household cats. Lane 12 is an FPV-positive sample from a household cat.

Discussion

The prevalence of FPV infection varies in many countries. In Saudi Arabia, FPLV serologic prevalence rates in wild cats, sand cats, and feral domestic cats are 5%, 0%, and 8%, respectively (21). FPV antibody prevalence in free-living European wild cats captured from 1991 to 1993 in central Spain was 18% (16). A cross-sectional survey of a convenient sample of domestic cats from a greater metropolitan area in Costa Rica was conducted to determine the prevalence of feline parvovirus antibodies. Antibodies against FPV were found in 92.8% of the cats sampled, but only 16.5% of them had been vaccinated (4). Antibodies against FPV (67%) have been detected in cats on the Galapagos Islands (14). The prevalence of FPV via PCR assay in our study was 2%. It was not possible to directly compare FPV prevalence among studies because different serologic methods were used to detect FPV. Some studies used an enzyme-linked immunosorbent assay (ELISA) to detect FPV antibody (3,4,14,16, 21). Most FPV prevalence studies employed antibody tests; however, reported prevalence may be artificially high due to antibodies present from vaccination or previous infections. Thus, because they identify current infections, antigen tests may offer more accurate detection and disease prognosis.

In this study, the FPV-positive rates in vaccinated and unvaccinated cats were 1.3% (1/77) and 2.3% (3/133), respectively. Previous studies have also reported a higher prevalence in unvaccinated cats (9,22). We also found that stray cats (3%) showed a higher FPV-positive rate than that in household cats (1%). The higher prevalence in stray cats may be because stray cats are generally exposed to high levels of environmental stress and are not typically vaccinated (16). Unvaccinated kittens that acquire maternal immunity though colostrum are protected for up to 3 months of age (13,23). Stray cats infected with FPV may also remain subclinical carriers after recovery; at least 80% of cats remain latently infected, and 29% of them shed virus spontaneously (1,5). These findings offer an explanation for the generally higher FPV-positive rates in stray cats than in client-owned or cattery breeding cats. Furthermore, most household cat owners manage their cats' health by vaccinating and preventing contact with infected cats. These results indicate that vaccination may prevent disease and increase the survival rate.

Sick cats are more likely to be infected than healthy cats (9,17). However, the prevalence of healthy cats was higher in our study. In addition, cats with FPV generally have leukopenia (11); however, we observed that only the single household cat with FPV had leukopenia (630/μL), whereas the leukocyte counts of the 3 strays with FPV were within the reference range (mean, 16,470/μL). Cats with normal leukocyte counts may have had leukopenia before testing or may have been tested before leukopenia occurred (1,5). Although cats were excluded if they were vaccinated against panleukopenia in the 3 weeks before admission, a vaccine-induced false-positive result for FPV cannot be totally ruled out in cats with an unknown vaccination history (1,5). Among the 4 positive samples, males (2 cats) and females (2 cats) were equally represented, and the breeds were 3 Korean Short Hairs and 1 Scottish Fold. No known sex or breed predilection exists for FPV infection, and all species of Felidae can become infected (6).

Our study demonstrates that FPV prevalence varies by region; higher rates of FPV infection were noted in Gangseo (1.5% in stray cats) and Gwangjin (0.5% in household cats). However, it was not possible to directly compare the prevalence of FPV infection in different areas because of differences in sample numbers and environmental characteristics of each region.

In conclusion, our study of stray and household cats is, to our knowledge, the first to survey FPV prevalence in Seoul. FPV testing is useful for epidemiological studies to detect infected animals and to prevent the spread of disease. In addition, we found that the FPV prevalence differed according to age and living environment of the cats. In asymptomatic cats, early detection of FPV antigen via PCR assay will increase survival rate and prevent the spread of disease.

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한국의 서울에 사는 길 고양이와 집 고양이에서 고양이 범백혈구감소증 바이러스의 유병률

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요 약 : 우리는 한국의 서울에 있는 다른 지역에 사는 길 고양이와 집 고양이의 범백혈구감소증 바이러스의 유병률을 조사하였다. 혈액 샘플은 200마리 고양이 (길 고양이 100마리와 집 고양이)에서 수집하였으며 모든 샘플은 중합효소 반응검사를 실시하였다. 전체적인 고양이 범백혈구감소증 바이러스의 유병률은 2%이다. 검사를 통해 양성 고양이 중 3% (100 분의 3)은 길 고양이이며 1% (100분의 1)은 집 고양이였다. 고양이 백혈구감소증 바이러스의 발생은 성묘 (1살이상, 0.5%) 보다 자묘 (1살이내, 1.5%)에서 더 높았다. 건강한 고양이의 양성률은 1.8% (3/166)이며 아픈 고양이의 양성률은 2.2% (1/44)이다. 예방 접종한 고양이의 양성률은 1.3% (1/77)이었고 예방 접종하지 않은 고양이의 양성률은 2.3% (3/133)이다. 항체검사와 비교시 고양이 범백혈구 바이러스 항원의 검출은 길 고양이와 집 고양이의 현재 질병 감염 상태를 암시할 수 있다. 따라서 항원검사는 질병의 진단과 치료에 도움을 줄 수 있다. 결론적으로 우리의 연구는 현재까지 서울에서 고양이 범백혈구감소증 바이러스의 유병률을 평가한 적이 없었기 때문에 비교적 중요하다. 고양이 범백혈구감소증 바이러스의 유병률은 길 고양이와 어린 고양이에서 높았다. 따라서 고양이 범백혈구감소증 바이러스 발생을 예방하기 위해서는 적절한 예방접종과 감시가 중요하다.

주요어 : 고양이 범백혈구 바이러스, 중합연쇄반응, 서울, 길 고양이, 집 고양이